this case, every presynapse in the preparation forms a direct contact on the recorded neuron. If every synapse has both AMPA and NMDA receptors, even a spillover response will always be accompanied by a direct response, i.e., a response with an AMPA component.

Gomperts et al. detect pure NMDA responses in this preparation in several ways. First, they note that evoked responses have a larger NMDAR component than spontaneous miniature responses that are selected based on having an AMPAR component. This suggests that a significant number of spontaneous events were not selected that have an NMDAR component and no AMPAR component. Furthermore, they are able to pick out spontaneous events that, when averaged, have a slow time course similar to that of a pure NMDAR response. These results strongly suggest that pure NMDA responses can be detected in this preparation and thus argue that there must be some mechanism, other than spillover, to account for pure NMDA responses.

Gomperts et al. examine this further: using immunolabeling techniques, they make two important observations. First, all presynaptic boutons have a cluster of adjacent postsynaptic receptors. Thus, indeed, any spillover response would also produce a direct response. Secondly, they show that a significant fraction of synaptic connections have NMDA and lack AMPA receptor immunolabeling and can thus account for the pure NMDAR transmission.

Thus, for this preparation, the authors argue that transmitter spillover cannot account for the pure NMDAR responses, and they provide anatomical evidence for synapses with only NMDARs. This, along with another recent study (Liao et al., 1999), indicates that cultured neuronal preparations have silent synapses that can be accounted for by synapses with only NMDARs. Such synapses have also been identified in the experimentally more hostile terrain of the intact brain with immunogold electron microscopy (Nusser et al., 1998; Petralia et al., 1999). Pure NMDAR synapses were found to be more prevalent in CA1 hippocampus early in postnatal development, supporting the view that initial synapses my be silent and become AMPAfied during development through an activity-dependent process (Nusser et al., 1998).

Finding that silent transmission can be due to action at synapses with only NMDARs enhances our knowledge about basic excitatory transmission in the brain. Furthermore, this provides an important element to a postsynaptic model for expression of LTP. These results come at the heel of several studies arguing against presynaptic changes during LTP. Three independent groups, using synaptic (Mainen et al., 1998) or peri-synaptic (Diamond et al., 1998; Lüscher et al., 1998) detectors of synaptic transmitter release, found no increase after LTP. While an optimistic observer may thus conclude that the tar is thinning, and that the LTC of LTP is getting resolved, there may (always) be more clever scenarios to consider.

Roberto Malinow
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Selected Reading

Diamond, J.S., Bergles, D.E., and Jahr, C.E. (1998). Neuron *21*, 425–433.

Gomperts, S.N., Rao, A., Craig, A.M., Malenka, R.C., and Nicoll, R.A. (1998). Neuron *21*, this issue, 1443–1451.

Isaac, J.T., Nicoll, R.A., and Malenka, R.C. (1995). Neuron *15*, 427–434.

Kullmann, D.M. (1994). Neuron *12*, 1111–1120.

Kullmann, D.M., and Asztely, F. (1998). Trends Neurosci. *21*, 8–14.

Liao, D., Jones, A., and Malinow, R. (1992). Neuron *9*, 1089–1097.

Liao, D., Hessler, N.A., and Malinow, R. (1995). Nature *375*, 400–404.

Liao, D., Zhang, X., O'Brien, R., Ehlers, M., and Huganir, R.L. (1999). Nat. Neurosci., in press.

Lüscher, C., Malenka, R.C., and Nicoll, R.A. (1998). Neuron *21*, 435–441.

Mainen, Z.F., Jia, Z., Roder, J., and Malinow, R. (1998). Nat. Neurosci. *7*, 579–586.

Nusser, Z., Lujan, R., Laube, G., Roberts, J.D., Molnar, E., Somogyi, P. (1998). Neuron *21*, 545–559.

Petralia, R.S., Esteban, J.A., Wang, Y.-X., Partridge, J.G., Zhao, H.-M., Wenthold, R.J., and Malinow, R. (1999). Nat. Neurosci., in press.

Eph Receptors, Ephrins, and PDZs Gather in Neuronal Synapses

Efficient intercellular communication depends on the localization of specific signaling proteins to particular sites on the cell surface. The synaptic junction, which mediates rapid communication between neurons, provides a striking example in which specific proteins accumulate at membrane specializations on both sides of the synapse. For instance, ionotropic glutamate receptors are highly concentrated in the postsynaptic membrane of excitatory synapses. What is the molecular mechanism underlying such localized clustering of membrane proteins? Recent studies have highlighted the role played by proteins that contain PDZ domains (Sheng, 1997; Ziff, 1997). PDZ domains are modular protein interaction domains that typically recognize short peptide sequences of four or more amino acids at the very C terminus of its ligands, and different PDZ domains recognize different C-terminal sequences. For example, PDZ domains in the PSD-95/SAP90 family of postsynaptic density proteins bind to the C-terminal -ESDV peptide sequence of NR2 subunits of the NMDA receptor. On the other hand, GluR2/3 subunits of AMPA receptors bind via their C termini (-SVKI) to GRIP, a protein containing seven PDZs (Dong et al., 1997). Studies of PDZ-based interactions in synapses have naturally focused on neurotransmitter receptors and ion channels, which are known to be concentrated in synaptic junctions. By contrast, little is known about receptor tyrosine kinases (RTKs) in neuronal synapses. Some RTKs (MuSK and erbB receptors) are concentrated in the vertebrate neuromuscular junction, but the mechanisms underlying this localization are unclear. No interactions between RTKs and PDZ domains have been reported in vertebrates. Enter Torres et al. (1998 [this issue of *Neuron*])

with two significant advances. First, they report that RTKs of the Eph family and their transmembrane ligands (ephrins) bind to specific PDZ domain proteins; second, certain Eph receptors and ligands are concentrated in neuronal synapses, probably in association with their PDZ binding partners.

Torres et al. show that the Eph RTK EphB2 (C-terminal sequence -SVEV) has specific affinity for PDZ domains in two different proteins, GRIP and PICK1, while EphA7 (-GIQV) can bind to GRIP, PICK1, and a third PDZ-containing protein, syntenin. Ligands belonging to the ephrin-B subfamily (-YYKV) also bind to GRIP, PICK1, and syntenin. The interaction between PDZ proteins and Eph receptors/ligands is not so surprising; after all, PDZ domains recognize just the last few amino acids of their ligands, and this C-terminal "zipcode" can be appended onto any class of protein. Indeed, the precedent for an interaction between an RTK (LET23) and a PDZ protein (LIN-7) has been established in *C. elegans* epithelial cells (Kaech et al., 1998). More unexpected is the ensuing finding that EphB2 and its ligand, Ephrin-B, are concentrated at synapses in cultured neurons, where their PDZ partners GRIP and PICK1 are also localized.

To date, Eph receptors and their ligands have been studied primarily in a developmental context. In the nervous system, these molecules are implicated in axon guidance, particularly in repulsion and in establishment of boundaries between groups of cells. What are Eph receptors and ephrins doing in synapses? It is tempting to speculate that they might be involved in synaptogenesis (like MuSK and erbB receptors) or in synaptic plasticity, perhaps by controlling the adhesion and/or repulsion of pre- and postsynaptic membranes. The synaptic localization of Eph receptors and their ligands needs to be confirmed in the brain and extended to other members of these protein families. Important questions will include whether the various Eph receptors and ephrins are differentially distributed among CNS synapses, and whether receptors and ligands are segregated to pre- and postsynaptic sides of the junction. Detailed analysis of mouse knockouts of Eph receptors and ephrins may shed more light on the roles of these proteins in synapses and in mature brain.

If it is early to speculate about the synaptic functions of Eph receptors and ephrins, what about the functional significance of their interactions with PDZ domain proteins? A prevailing idea is that the PDZ protein is important for the subcellular localization of its binding partners. In *Drosophila*, the PSD-95 homolog Discs-large is localized in synapses and is essential for the synaptic clustering of its PDZ interactors, Shaker and Fasciclin II (Thomas et al., 1997; Zito et al., 1997). Genetic studies on InaD (in *Drosophila*) and LIN-2/LIN-7/LIN-10 (in *C. elegans*) additionally support the idea that PDZ-mediated interactions are important for the subcellular targeting of the interacting proteins, both at synapses and at other specialized membrane domains (Tsunoda et al., 1997; Kaech et al., 1998; Rongo et al., 1998). By analogy, EphB2 and ephrin-B1 localization in neuronal synapses may depend on their binding to synaptic PDZ proteins like GRIP and PICK1.

Another (not mutually exclusive) concept is that PDZ proteins have a scaffolding function and can assemble a specific protein complex around their membrane protein ligands. In *Drosophila* photoreceptors, a physiologically coupled "transducisome" of phototransduction signaling proteins is built around InaD, a protein with five PDZs (Tsunoda et al., 1997). In synapses, PSD-95 can assemble a specific cytoskeletal-signaling complex that is physically linked to the NMDA receptor (Craven and Bredt, 1998). Perhaps the interaction of Eph receptors and ligands with PDZ proteins couples them to intracellular signaling networks or modulatory enzymes. This may be particularly significant for the ephrin-B ligands, which participate in reciprocal signaling with their Eph receptors despite lacking a catalytic domain. PICK1 has only one PDZ domain but was previously identified as a protein kinase C (PKC)-binding protein (Staudinger et al., 1997); thus, PICK1 could mediate the association of PKC with specific Eph receptors and ligands. PICK1 also appears to be a direct substrate for the Eph RTK (Torres et al., 1998). GRIP has seven PDZs and the potential to scaffold an elaborate protein architecture around Eph receptors and their ligands. Since GRIP was originally identified as an AMPA receptor–binding protein, it will be interesting to determine whether Eph receptors or ligands are physically and functionally coupled to AMPA receptors in synapses. To date, there has been little evidence for regulation of AMPA receptors by tyrosine phosphorylation.

Unlike many ligands of RTKs, Eph ligands are not active as soluble proteins; ephrins need to be clustered on the cell surface for them to stimulate their cognate Eph receptors. It is pertinent, therefore, that surface aggregation of transmembrane proteins is a common outcome of interaction with PDZ proteins. The ability of certain PDZ proteins to cluster their binding partners may reflect the propensity of PDZ-containing proteins to multimerize and/or their ability to bind these membrane proteins in a multivalent manner. Indeed, PICK1 can aggregate ephrin-B1 in heterologous cells (Torres et al., 1998). Clustering by PICK1 or GRIP may optimize the presentation of ephrins to their Eph receptors in vivo; such a mechanism offers another potential level for regulation of Eph signaling. PDZ-dependent clustering of Eph receptors and ligands at specific subcellular sites (e.g., in growth cones) may also be important for Eph/ephrin function in development. Thus, following up the findings of Torres et al. promises to shed new light on the functions and mechanisms of the Eph system in both developing and mature brain.

Yi-Ping Hsueh and Morgan Sheng
Howard Hughes Medical Institute and Department of Neurobiology
Massachusetts General Hospital and
Harvard Medical School
Boston, Masachusetts 02114

Selected Reading

Craven, S.E., and Bredt, D.S. (1998). Cell *93*, 495–498.

Dong, H., O'Brien, R.J., Fung, E.T., Lanahan, A.A., Worley, P.F., and Huganir, R.L. (1997). Nature *386*, 279–284.

Kaech, S.M., Whitfield, C.W., and Kim, S.K. (1998). Cell *94*, 761–771.

Rongo, C., Whitfield, C.W., Rodal, A., Kim, S.K., and Kaplan, J.M. (1998). Cell *94*, 751–759.

Sheng, M. (1997). Nature *386*, 221–223.

Staudinger, J., and Olsen, E.N. (1997). J. Biol. Chem. *272*, 32019–32024.

Thomas, U., Kim, E., Kuhlendahl, S., Ho Koh, Y., Gundelfinger, E.D., Sheng, M., Garner, C.C., and Budnik, V. (1997). Neuron *19*, 787–799.

Torres, R., Firesrein, B.L., Dong, H., Staudinger, J., Olsen, E.N., Huganir, R.L., Bredt, D.S., Gale, N.W., and Yancopoulos, G.D. (1998). Neuron *21*, this issue, 1453–1463.

Tsunoda, S., Sierralta, J., Sun, Y., Bodner, R., Suzuki, E., Becker, A., Socolich, M., and Zuker, C.S. (1997). Nature *388*, 243–249.

Ziff, E.B. (1997). Neuron *19*, 1163–1174.

Zito, K., Fetter, R.D., Goodman, C.S., and Isacoff, E.Y. (1997). Neuron *19*, 1007–1016.